CLAIMS

What is claimed is:

1. A method for detecting a target nucleic acid molecule or target nucleic acid molecular complex comprising:

(a) contacting two or more probes complementary to the molecule or molecular complex, said molecule or molecular complex being labeled with one or more fluorescent dye molecules of the same dye or labeled with two dyes that are indistinguishable by their emission characteristics in an assay instrument, wherein each probe interacts specifically with a different target nucleic acid sequence or a structure on the molecule or molecular complex; and

(b) detecting interaction of the probes with the molecule or molecular complex, said interaction being detected by an increase in fluorescence intensity during a detection interval having a fluorescence intensity above the fluorescence intensity of any individual free probe, wherein molecule or molecular complex is analyzed such that only individual molecules or molecular complexes in contact with a probe are within an interrogation volume and within a detection time interval.

2. A method according to claim 1, wherein each probe in contact with the molecule or molecular complex possesses substantially equal fluorescence intensity.

3. A method according to claim 1, wherein each probe in contact with the molecule or molecular complex has a fluorescence intensity distinguishable from another probe in contact with the same molecule or molecular complex.

4. A method according to claim 1, wherein unbound probes are removed from contacting action (a) prior to detection.

5. A method according to claim 1, wherein during detection action (b) the concentration of unhybridized probes is maintained at a level such that the fluorescence intensity of unhybridized probes can be distinguished from the fluorescence intensity of the probes in contact with the molecule or molecular complex.

6. A method according to claim 1, wherein the probes are selected from the group consisting of DNA, RNA, PNA, LNA, XLNT, nucleic acid sequence-specific molecules, structure-specific molecules, and any combination thereof.

7. A method according to claim 1, wherein detection of probes in contact with the molecule or molecular complex is provided by a single fluorescence detector.

8. A method according to claim 1, wherein detection of probes in contact with the molecule or molecular complex is provided by two or more fluorescence detectors.

9. A method according to claim 1, wherein detection of probes in contact with the molecule or molecular complex is provided by a CCD.

10. A method for detecting a target nucleic acid molecule or target nucleic acid molecular complex comprising:

(a) contacting two or more probes complementary to the molecule or molecular complex, said molecule or molecular complex being labeled with one or more fluorescent dye molecules of the same dye or labeled with two dyes that are indistinguishable by their emission characteristics in an assay instrument, wherein each probe interacts specifically with a different target nucleic acid sequence or a structure on the molecule or molecular complex; and

(b) detecting interaction of the probes in contact with the molecule or molecular complex, said interaction being detected by an increase in fluorescence intensity during a detection interval having a fluorescence intensity above the fluorescence intensity of any individual free probe; and

(c) detecting the velocity of the probes in contact with molecule or molecular complex, in relation to an expected velocity for such a complex in a transport tube, wherein the velocity is imparted on the probes in contact with the molecule or molecular complex by pumping the probes in contact with the molecule or molecular complex through the tube or by applying an electric field to the probes in contact with the molecule or molecular complex.

11. A method according to claim 10, wherein the number of probes interacting with the molecule or molecular complex can be ascertained by measuring the fluorescence intensity during a detection interval together with the velocity of the probes in contact with the molecule or molecular complex in the transport tube.

12. A method according to claim 10, wherein the specific probes interacting with the molecule or molecular complex can be ascertained by measuring fluorescence intensity during a detection interval together with the velocity of the probes in contact with the molecule or molecular complex in a transport tube.

13. A method according to claim 10, wherein each probe in contact with the molecule or molecular complex possesses substantially equal fluorescence intensity.

14. A method according to claim 10, wherein each probe in contact with the molecule or molecular complex has a fluorescence intensity distinguishable from another probe in contact with the same molecule or molecular complex.

15. A method according to claim 10, wherein unbound probes are removed from contacting action (a) prior to detection.

16. A method according to claim 10, wherein during detection actions (b) and (c) the concentration of unhybridized probes is maintained at a level such that the fluorescence intensity of unhybridized probes can be distinguished from the fluorescence intensity of the probes in contact with the molecule or molecular complex.

17. A method according to claim 10, wherein the probes are selected from the group consisting of DNA, RNA, PNA, LNA, XLNT, nucleic acid sequence-specific molecules, structure-specific molecules, and any combination thereof.

18. A method according to claim 10, wherein detection of probes in contact with the molecule or molecular complex is provided by a single fluorescence detector.

19. A method according to claim 10, wherein detection of probes in contact with the molecule or molecular complex is provided by two or more fluorescence detectors.

20. A method according to claim 10, wherein detection of probes in contact with the molecule or molecular complex is provided by a CCD.

21. A method for detecting a target nucleic acid molecule or target nucleic acid molecular complex comprising:

(a) contacting two or more probes complementary to the molecule or molecular complex, said molecule or molecular complex being labeled with one or more fluorescent dye molecules of the same dye or labeled with two dyes that are indistinguishable by their emission characteristics in an assay instrument, wherein each probe interacts specifically with a different target nucleic acid sequence or a structure on the molecule or molecular complex; and

(b) detecting interaction of the probes with the molecule or molecular complex, said interaction being detected by a change during a detection interval in a fluorescence parameter selected from the group consisting of fluorescence lifetime, fluorescence polarization or FRET, wherein molecule or molecular complex is analyzed such that only individual molecules or molecular complexes in contact with a probe are within an interrogation volume and within a detection time interval.

22. A method according to claim 21, wherein the specific probes interacting with the target can be ascertained by the change in a fluorescence parameter during a detection interval.

23. A method according to claim 21, wherein the specific probes interacting with the target can be ascertained by the change in a fluorescence parameter during a detection interval together with the velocity of the target-probes complex in a transport tube, such molecular velocity imparted by pumping of sample through the tube or by application of an electric field to the sample

24. A method according to claim 21, wherein each probe in contact with the molecule or molecular complex possesses substantially equal fluorescence parameter values.

25. A method according to claim 21, wherein each probe in contact with the molecule or molecular complex possesses a fluorescence parameter value different from another probe in contact with the same molecule or molecular complex.

26. A method according to claim 21, wherein unbound probes are removed from contacting action (a) prior to detection.

27. A method according to claim 21, wherein during detection action (b) the concentration of unhybridized probes is maintained at a level such that the fluorescence intensity of unhybridized probes can be distinguished from the fluorescence intensity of the probes in contact with the molecule or molecular complex.

28. A method according to claim 21, wherein the probes are selected from the group consisting of DNA, RNA, PNA, LNA, XLNT, nucleic acid sequence-specific molecules, structure-specific molecules, and any combination thereof.

29. A method according to claim 21, wherein detection of probes in contact with the molecule or molecular complex is provided by a single fluorescence detector.

30. A method according to claim 21, wherein detection of probes in contact with the molecule or molecular complex is provided by two or more fluorescence detectors.

31. A method according to claim 21, wherein detection of probes in contact with the molecule or molecular complex is provided by a CCD.

32. A method according to claim 1, wherein the number of probes interacting with the molecule or molecular complex can be ascertained by measuring the fluorescence intensity during a detection interval

33. A method according to claim 1, wherein the specific probes interacting with the molecule or molecular complex can be ascertained by measuring fluorescence intensity during a detection interval

32. A method for detecting a target nucleic acid molecule or target nucleic acid molecular complex comprising:

(a) contacting two or more probes complementary to the molecule or molecular complex, said molecule or molecular complex being labeled with one or more fluorescent dye molecules of the same dye or labeled with two dyes that are indistinguishable by their emission characteristics in an assay instrument, wherein each probe interacts specifically with a different target nucleic acid sequence or a structure on the molecule or molecular complex; and

(b) detecting interaction of the probes in contact with the molecule or molecular complex, said interaction being detected by a change during a detection interval in a fluorescence parameter selected from the group consisting of fluorescence lifetime, fluorescence polarization or FRET, during a detection interval having a fluorescence intensity above the fluorescence intensity of any individual free probe; and

(c) detecting the velocity of the probes in contact with molecule or molecular complex, in relation to an expected velocity for such a complex in a transport tube, wherein the velocity is imparted on the probes in contact with the molecule or molecular complex by pumping the probes in contact with the molecule or molecular complex through the tube or by applying an electric field to the probes in contact with the molecule or molecular complex.

33. A method according to claim 32, wherein the specific probes interacting with the target can be ascertained by the change in a fluorescence parameter during a detection interval.

34. A method according to claim 32, wherein the specific probes interacting with the target can be ascertained by the change in a fluorescence parameter during a detection interval together with the velocity of the target-probes complex in a transport tube, such molecular velocity imparted by pumping of sample through the tube or by application of an electric field to the sample

35. A method according to claim 32, wherein each probe in contact with the molecule or molecular complex possesses substantially equal fluorescence parameter values.

36. A method according to claim 32, wherein each probe in contact with the molecule or molecular complex possesses a fluorescence parameter value different from another probe in contact with the same molecule or molecular complex.

37. A method according to claim 32, wherein unbound probes are removed from contacting action (a) prior to detection.

38. A method according to claim 32, wherein during detection actions (b) and (c) the concentration of unhybridized probes is maintained at a level such that the fluorescence intensity of unhybridized probes can be distinguished from the fluorescence intensity of the probes in contact with the molecule or molecular complex.

39. A method according to claim 32, wherein the probes are selected from the group consisting of DNA, RNA, PNA, LNA, XLNT, nucleic acid sequence-specific molecules, structure-specific molecules, and any combination thereof.

40. A method according to claim 32, wherein detection of probes in contact with the molecule or molecular complex is provided by a single fluorescence detector.

41. A method according to claim 32, wherein detection of probes in contact with the molecule or molecular complex is provided by two or more fluorescence detectors.

42. A method according to claim 32, wherein detection of probes in contact with the molecule or molecular complex is provided by a CCD.

43. A method for determining the number of probes interacting with a target comprising:

(a) contacting two or more probes that interact specifically with the target, said target being labeled with one or more fluorescent dye molecules of the same dye or labeled with two dyes that are indistinguishable by their emission characteristics in an assay instrument, wherein each probe interacts specifically with a different target nucleic acid sequence or a structure on the molecule or molecular complex, and wherein fluorescent intensity of each probe is equal to that of other probes within detection capabilities of an instrument used for the detection; and

(b) detecting interaction of the probes in contact with the target, said interaction being during a detection interval that is above the fluorescence intensity for any individual free probe molecule;

(c) detecting the velocity of the probes in contact with the target, in relation to an expected velocity for such a complex in a transport tube, wherein the velocity of the molecular probes-target hybrids matches the expected velocity for such a complex in a transport tube, and wherein the velocity is imparted on the probes in contact with the molecule or molecular complex by pumping the probes in contact with the molecule or molecular complex through the tube or by applying an electric field to the probes in contact with the molecule or molecular complex; and

(d) determining the number of probes interacting with each target molecule by dividing the fluorescent intensity detected per detection time interval by the unit intensity per detection time interval

44. A method according to claim 43, wherein the number of free probe molecules (n) in the interrogation volume is greater than one, and the number of probes (greater than n) interacting with each target is determined by measuring the fluorescence intensity per detection time interval (I) and calculating the quantity (I/U)-n.

45. A method according to claim 43, wherein when the probes are labeled with luminescent dye(s) and the number of probes interacting with the target is be ascertained by the change in a luminescent parameter during a detection interval.

46. A method according to claim 45, wherein the luminescent parameter is selected from the group consisting of luminescence intensity, luminescence spectral distribution, burst size, burst duration, fluorescence lifetime, fluorescence polarization, FRET, and any combination thereof.

47. A method according to claim 45, wherein when the probes are labeled with luminescent dye(s) and the number of probes interacting with the target is ascertained by the change in a luminescent parameter during a detection interval together with the velocity of the target-probes complex in a transport tube, such molecular velocity imparted by pumping of sample through the tube or by application of an electric field to the sample.

48. A method according to claim 47, wherein the luminescent parameter is selected from the group consisting of luminescence intensity, fluorescence lifetime, fluorescence polarization, FRET, and any combination thereof.

49. A method according to claim 43, wherein the target is a nucleic acid and the probes are selected from the group consisting of nucleic acids, PNAs, LNAs, XLNT probes, peptides, proteins, small molecules, and any combination thereof.

50. A method for detecting a target nucleic acid molecule or target nucleic acid molecular complex comprising:

(a) contacting two or more probes that interact specifically with the target, said probe species being labeled with one or more fluorescent dye molecules of the same dye or labeled with one or more dye molecules of at least one other dye that is indistinguishable from the first dye by their emission characteristics in the assay instrument, wherein probe labeling takes place to the extent that luminescent or fluorescent intensity per detection time unit of each probe

species is unique, and wherein each probe species is differentiable from the other probe species in the assay based on luminescent or fluorescent intensity per detection time unit; and

(b) detecting interaction of the probes in contact with the target, such that only individual target or probe molecules are within an interrogation volume and within a detection time interval and no more than one probe molecule is in the interrogation volume within a detection time interval, wherein specific probe species with target is detected by an increase in fluorescence intensity during a detection interval that is equal to the additive fluorescence intensities of more than one probe species; or

(c) detecting interaction of the probes in contact with the target, such that only individual target or probe molecules are within an interrogation volume and within a detection time interval and no more than one probe molecule is in the interrogation volume within a detection time interval, wherein specific multiple probe species with target is detected by an increase in fluorescence intensity during a detection interval that is equal to the additive fluorescence intensities of more than one probe species and the velocity of the molecular probes-target hybrids matches the expected velocity for such a complex in a transport tube, such molecular velocity imparted by pumping of sample through the tube or by application of an electric field to the sample.

51. A method according to claim 50, wherein the probes are labeled with luminescent dye(s) and the number of probes interacting with the target is ascertained by the change in a luminescent parameter during a detection interval.

52. A method according to claim 51, wherein the luminescent parameter is selected from the group consisting of luminescence intensity, luminescence spectral distribution, burst size, burst duration, fluorescence lifetime, fluorescence polarization, FRET, and any combination thereof.

53. A method according to claim 50, wherein the probes are labeled with luminescent dye(s) and the number of probes interacting with the target is ascertained by the change in a luminescent parameter during a detection interval together with the velocity of the target-probes complex in a transport tube, such molecular velocity imparted by pumping of sample through the tube or by application of an electric field to the sample

54. A method according to claim 53, wherein the luminescent parameter is selected from the group consisting of luminescence intensity, luminescence spectral distribution, burst size, burst duration, fluorescence lifetime, fluorescence polarization, FRET and any combination thereof.

55. A method according to claim 50, wherein the target is a nucleic acid and probes are selected from the group consisting of nucleic acids, PNAs, LNAs, XLNT probes, peptides, proteins, small molecules, and any combination thereof.